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Evaluation of maternal parent and puroindoline allele on kernel texture in a reciprocal cross between two hard spring wheat cultivars

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Summary

Kernel texture is an important characteristic for both the milling and the end-use quality of wheat (*Triticum aestivum* L.). Gene sequence variation and mutations to the two puroindoline genes (*Pina* and *Pinb*), located at the *Ha* locus on chromosome 5DS, account for the majority of variation in wheat kernel texture. Other factors also influence kernel texture, including effects associated with different maternal parent backgrounds. To investigate the effect of two hard puroindoline alleles in different maternal backgrounds, a population of 228 recombinant inbred lines (RILs) derived from a reciprocal cross between two wheat cultivars 'ID377s' (*Pina-D1b*/*Pinb-D1a*) and 'Klasic' (*Pina-D1a*/*Pinb-D1b*) were examined in two succeeding generations (F_7 & F_8). Kernel texture was determined using the Single Kernel Characterization System (SKCS) and the RIL puroindoline haplotype was identified by the sequence-specific PCR amplification of each gene. Analysis of variance identified a significant ($P \leq 0.001$) effect of the maternal parent and puroindoline mutation on kernel texture. RILs containing the *Pina-D1b* mutation were significantly harder than lines containing the *Pinb-D1b* mutation. RILs which had Klasic as the maternal parent were significantly harder than those which had ID377s as the maternal parent. When the maternal parent and puroindoline allele were analyzed in combination, RILs derived from Klasic as the maternal parent and the *Pina-D1b* allele were significantly harder ($P \leq 0.001$) than those containing the same allele but ID377s as the maternal parent. The same occurred for RILs containing the *Pinb-D1b* allele, lines with Klasic as the maternal parent were harder than lines with ID377s as the maternal parent. These results corroborate the harder phenotype of the *Pina-D1b* allele and indicate a significant maternally-inherited contribution to kernel texture variation.

Abbreviations: SKCS: single kernel characterization system; SSD: single seed descent

Introduction

Kernel texture is one of the primary end use quality characteristics of cultivated wheat (*Triticum aestivum* L.). The classification of wheat into either the 'hard' or 'soft' class is based upon the texture of the mature endosperm. This classification forms the fundamental basis of differentiation of wheat for world trade, milling and end-use properties (reviewed in Pomeranz & Williams, 1990; Morris, 2002). The texture of hard and soft wheat is controlled predominantly by the hardness locus (*Ha*) located on the distal end of chromosome 5DS (Morris, 2002). A marker protein, friabilin, was identified by Greenwell & Schofield (1986) for grain softness. The friabilin protein was later determined to be composed primarily of two major polypeptides, designated puroindoline a and puroindoline b. The respective genes (*Pina* and *Pinb*) coding for these polypeptides were found to be tightly linked to the *Ha* locus (Jolly et al., 1993; Sourdille et al., 1996).

Specific mutation to either the *Pina* or *Pinb* gene results in the expression of a hard phenotype. Seven hard alleles of puroindoline b and one hard allele in puroindoline a have been identified. All nucleotide changes in *Pinb* are single nucleotide substitutions; four result in amino acid substitutions and three result in 'stop' codons (Morris, 2002; Pan et al., 2004). The exact mutation that nullifies the action of the *Pina* gene, resulting in the complete absence of the puroindoline a protein, is as yet unknown. Of these hard alleles two are most prevalent in the North American gene pool: the *Pina* 'null' allele (*Pina-D1b*) and the *Pinb* position 46 amino acid substitution of glycine to serine (*Pinb-D1b*) (Morris et al., 2001). Giroux et al. (2000) observed that $F_{3:6}$ progeny segregating for *Pina-D1b* and *Pinb-D1b* were significantly different in hardness in only one of three different crosses, where *Pina-D1b* was harder. Martin et al. (2001) compared a set of recombinant inbred lines (RILs) derived from a *Pina-D1b*/*Pinb-D1b* cross. In this population, the *Pina-D1b* RILs were significantly harder.

Puroindoline gene mutation accounts for most of the variation in grain endosperm texture between hard and soft wheat varieties (Campbell et al., 1999; Martin et al., 2001; Lillemo & Ringlund, 2002). Factors contributing to the residual variation in kernel texture have not been well defined. Due to the tight linkage and sequence similarity to the puroindoline genes, it is plausible that grain softness protein (GSP) might be associated with endosperm texture. However, no specific relationship between GSP and endosperm texture has been identified (Morris, 2002; Tranquilli et al., 2002). Pentosans (primarily arabinoxylans and arabinogalactans) were found to have a significant modifying effect on endosperm texture, especially in soft wheat (Bettge & Morris, 2000). Quantitative trait loci (QTL) analysis has identified specific regions of the wheat genome that are not linked to the *Ha* locus and are associated with endosperm texture. These QTLs have been identified on chromosomes 2A, 2DL and 6B (Campbell et al., 1999), and 1A and 6D (Perretant et al., 2000).

This research examines a recombinant inbred line (RIL) population from a reciprocal cross between the two hard white spring wheat cultivars 'ID377s' (*Pina-D1b*/*Pinb-D1a*) and 'Klasic' (*Pina-D1a*/*Pinb-D1b*). The RIL population was partitioned according to each line's maternal parent and puroindoline allele. The SKCS kernel hardness of the RILs was analyzed across two advanced generations to determine the effect of the puroindoline genes, the maternal parent and their interaction on kernel texture.

Materials and methods

Plant material

A population of 228 recombinant inbred lines (RILs) was produced from a reciprocal cross between the hard white spring wheat cultivars 'ID377s' (*Pina-D1b*/*Pinb-D1a*) and 'Klasic' (*Pina-D1a*/*Pinb-D1b*). Lines were advanced by single seed descent (SSD) to the F_7 generation. Endosperm texture was assessed in the F_6 generation, and in the succeeding F_7 generation (F_7 and F_8 grain, respectively). Individual plants were grown to maturity in a greenhouse using supplemental lighting and common cultural practices. The parental lines were also grown with each generation for comparison.

Kernel texture

The kernel texture of the two generations of 228 lines and parents was assessed with the Single Kernel Characterization System 4100 (SKCS) (Perten Instruments North America, Inc., Springfield, IL, U.S.A.). SKCS hardness values were produced from crushing a sample of 100 kernels from each line.

Puroindoline allele characterization

Puroindoline allele characterization was performed on the F_7 generation. DNA was extracted from seeds using the technique described by Guidet et al. (1991). Puroindoline genes were amplified using the protocols and sequence-specific primers of Giroux & Morris (1997) and Gautier et al. (2000). Two sequence-specific primers for the *Pinb-D1a* and *Pinb-D1b* alleles (Giroux & Morris, 1997) were used, one of which targets the single nucleotide mutation which confers the position 46 glycine-serine substitution of *Pinb-D1b*, and a primer for the wild type. The sequence-specific primers of Gautier et al. (2000) do not amplify *Pina-D1b*, producing a null result. Each line was amplified one or more times to confirm the presence or absence of the wild type of puroindoline a (*Pina-D1a*), the presence or absence of the wild type puroindoline b (*Pinb-D1a*) or the glycine-serine mutation of puroindoline b (*Pinb-D1b*). Because of the tight linkage between the puroindoline genes (Giroux & Morris, 1997; Tranquilli et al., 1999; Martin et al., 2001), the presence of one altered gene (*Pina-D1b* or *Pinb-D1b*) indicated the presence of the wild type puroindoline allele in the other corresponding gene. Both puroindoline genes were amplified in each line; the absence of puroindoline a product

in lines containing the *Pina-D1b* allele was confirmed by identifying the wild type allele (*Pinb-D1a*) in the corresponding puroindoline b gene.

Statistical analysis

Hardness scores produced by testing lines with the SKCS were analyzed using the general linear model (GLM) approach to analysis of variance (SAS ver8.2, Cary, NC, U.S.A.). Analysis of variance was conducted using a model incorporating the factors puroindoline mutation, generation of growth, and maternal parent. The two generations were treated as blocks since they were grown at different times. Maternal parent and puroindoline allele were considered fixed effects; mean square error was used to test all other model components.

Data were examined to determine if transgressive segregation occurred when the population was classified according to either the maternal parent or the hard allele. Transgressive segregation from the maternal parent was defined as lines that were either greater than the highest ranking parent or less than the lowest ranking parent by more than one LSD ($\alpha = 0.05$). Transgressive segregation from the hard allele was defined as lines differing from the mean of each allele by more than one LSD.

Results

Puroindoline classification and population structure

Puroindoline genes were amplified and characterized in all 228 lines and parents. The parental lines, ID377s and Klasic, were confirmed as possessing the *Pina-D1b*/*Pinb-D1a* and *Pina-D1a*/*Pinb-D1b* haplotypes, respectively. All RILs contained only one hardness mutation, no RIL contained both.

Of the 144 RILs with ID377s as the maternal parent, 94 contained the *Pina-D1b* allele and 50 contained the *Pinb-D1b* allele. Of the 84 RILs with Klasic as the maternal parent, 48 contained the *Pina-D1b* allele and 36 contained the *Pinb-D1b* allele. Although no particular control was applied to the advancement of the SSD generations, how closely this population conforms to expected allele frequencies was examined. Across the two parental types, there was a significant ($P = 0.01$) deviation from the expected 1:1 frequency of *Pina-D1b* versus *Pinb-D1b* alleles ($\chi^2 = 15.8$), with a higher than expected frequency of the *Pina-D1b* allele. This result was attributable to the frequency of alleles among the lines with ID377s as the maternal parent, as the frequency of puroindoline alleles among the lines with Klasic as the maternal parent did not deviate significantly from expected ($\chi^2 = 1.7$).

SKCS hardness

SKCS hardness values for the RIL population over both generations ranged from 50.8 to 96.5, with a mean value of 71.8 (data not shown). The standard deviation was consistent across the range of hardness values and ranged from 8.9 to 17.8. The hardness value of the parental cultivar ID377s (78.9) containing the *Pina-D1b* allele was significantly ($P \leq 0.001$) harder than that of the other parent, Klasic (72.4), containing the *Pinb-D1b* allele (analysis not shown). Similar, though not significant, differences were observed by Martin et al. (2001), where the parent of a RIL population containing *Pina-D1b* (Butte 86) tended to have a harder kernel texture, as measured by near-infrared reflectance (NIR), than the other parent (ND 2603) containing *Pinb-D1b*.

Lines with ID377s as their maternal parent ranged in SKCS hardness values from 50.8 to 92.4 with a mean value of 70.1, whereas those lines with Klasic as the maternal parent ranged in SKCS hardness values from 55.0 to 96.5 with a mean value of 74.6 (Figure 1). Lines containing the *Pina-D1b* allele ranged in SKCS hardness values from 54.7 to 96.5 with a mean value of 73.6, whereas those lines containing the *Pinb-D1b* allele ranged in SKCS hardness from 50.8 to 89.8 with a mean value of 68.6 (Figure 2). Since both distributions exhibit considerable over-lap, ANOVA was applied to test for mean differences between maternal parents and puroindoline allele.

Analysis of variance of kernel texture

Both the maternal parent and the puroindoline allele were significant ($P \leq 0.001$) in their association with variation in endosperm texture, however their interaction was not (Table 1). This model accounted for a modest amount of the variation ($R^2 = 0.24$) observed in this population and is typical of that observed in hard wheat/hard wheat crosses (Giroux et al., 2000; Martin et al., 2001).

ANOVA indicated that the blocking factor was significant ($P \leq 0.001$) (Table 1). As the generations of this RIL population were highly advanced, this indicated that the two greenhouse environments

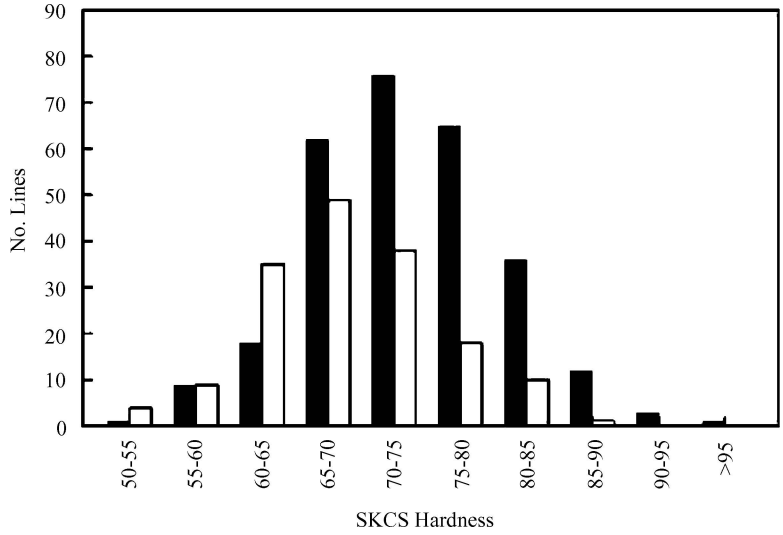

Figure 1. Distribution of SKCS hardness of RIL lines from the F_7 and F_8 generations derived from a reciprocal crosses of ID377s and Klasic hard white spring wheat cultivars. Black bars represent RILs containing *Pina-D1b*, hollow bars represent RILs containing *Pinb-D1b*.

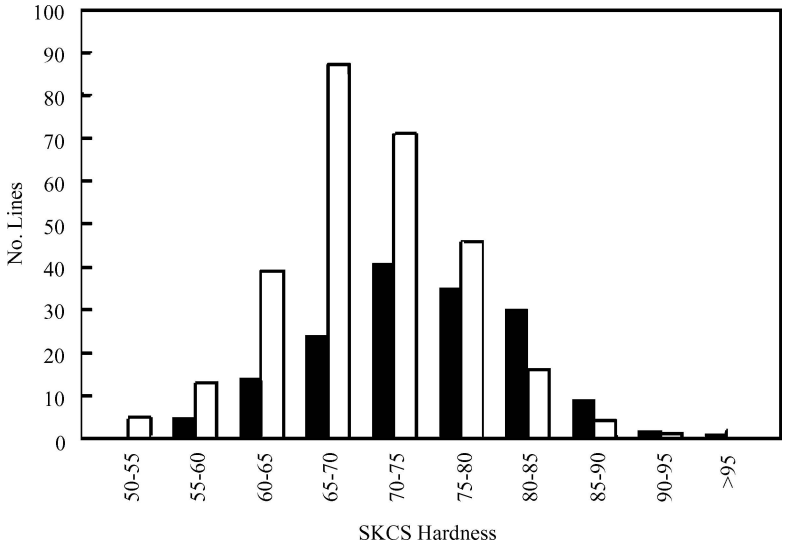

Figure 2. Distribution of SKCS hardness of RIL lines from the F_7 and F_8 generations derived from a reciprocal crosses of ID377s and Klasic hard white spring wheat cultivars. Black bars represent RILs with a maternal parent of Klasic, hollow bars represent RILs with a maternal parent of ID377s.

were slightly different. However, the Type III mean square for block was considerably less than either of the main effects (maternal parent or puroindoline allele) (Table 1). ANOVA demonstrated that RILs with Klasic (74.6) as the maternal parent were significantly harder than those with ID377s (70.1) as the maternal parent (Table 2). Furthermore, ANOVA identified the *Pina-D1b* puroindoline allele (73.6) as being significantly harder than the *Pinb-D1b* allele (68.6) (Table 3). These results are similar to those previously observed (Giroux et al., 2000; Martin et al., 2001). Transgressive segregation was observed in both directions for the maternal parental type and the puroindoline allele. Transgressive segregation for kernel texture was also observed by both Martin et al. (2001) and Campbell et al. (1999).

Since the preceding ANOVA indicated no significant interaction between maternal parent and puroindoline alleles, the four parent-allele combinations were examined. The population was re-classified using the

Table 1. ANOVA for the effect of the whole model, maternal parent, puroindoline allele and their interaction on the kernel texture of a RIL population derived from a reciprocal cross between ID377s (*Pina-D1b*/*Pinb-D1a*) and Klasic (*Pina-D1a*/*Pinb-D1b*)

Source	df	Mean square	*F* value	Pr > *F*
Whole model	3	1452	34.0	<0.0001
Maternal parent (M)	1	2471	57.8	<0.0001
Puroindoline allele (P)	1	2632	61.5	<0.0001
Interaction (MxP)	1	46	1.1	0.30
Block	1	825	19.3	<0.0001
Error	438	43	–	–

Table 2. Mean kernel texture of a RIL population derived from a reciprocal cross between ID377s (*Pina-D1b*/*Pinb-D1a*) and Klasic (*Pina-D1a*/*Pinb-D1b*) classified according to maternal parent

Maternal parent	Mean SKCS hardness[a]	Number of RILs
ID377s	70.1 a	288
Klasic	74.6 b	168
$LSD_{(0.05)}$	1.3	

[a]Means followed by the same letter are not significantly different as determined by LSD ($\alpha = 0.05$).

Table 3. Mean kernel texture of a RIL population derived from a reciprocal cross between ID377s (*Pina-D1b*/*Pinb-D1a*) and Klasic (*Pina-D1a*/*Pinb-D1b*) classified according to puroindoline allele

Puroindoline allele	Mean SKCS hardness[a]	Number of RILs
Pina-D1b	73.6 a	284
Pinb-D1b	68.6 b	172
$LSD_{(0.05)}$	1.3	

[a]Means followed by the same letter are not significantly different as determined by LSD ($\alpha = 0.05$).

combination of the maternal parent and the puroindoline allele, rather than examining the main effects independently. This reclassification created four sub-populations. The combination of the maternal parent and puroindoline allele was significant (Table 4), and accounted for a similar amount of the variation in endosperm texture as the individual main effects model ($R^2 = 0.23$). The maternal parent/puroindoline allele classification sub-populations could be distinctly separated into three groups using Duncan's multiple range test (DMRT) (Table 5). RILs with Klasic as the maternal parent that possessed the *Pina-D1b* allele were the hardest, with a mean SKCS hardness value of 76.5. The second DMRT group contained two sub-populations, RILs with ID377s as the maternal parent and containing

Table 4. ANOVA for the effect of the combination of maternal parent and puroindoline allele on the kernel texture of a RIL population derived from a reciprocal cross between ID377s (*Pina-D1b*/*Pinb-D1a*) and Klasic (*Pina-D1a*/*Pinb-D1b*)

Source	Df	Mean square	*F* value	Pr > *F*
Combination	3	1685	39	<0.0001
Block	1	825	19	<0.0001
Error	438	43	–	–

Table 5. Mean kernel texture of a RIL population derived from a reciprocal cross between ID377s (*Pina-D1b*/*Pinb-D1a*) and Klasic (*Pina-D1a*/*Pinb-D1b*) classified according to combinations of maternal parent and puroindoline allele

Parent/ puroindoline Allele	Mean SKCS hardness[a]	Number of RILs
Klasic/*Pina-D1b*	76.6 a	96
ID377s/*Pina-D1b*	72.1 b	188
Klasic/*Pinb-D1b*	71.9 b	72
ID377s/*Pinb-D1b*	66.3 c	100

[a]Means followed by the same letter are not significantly different as determined by Duncan's Multiple Range Test ($\alpha = 0.05$).

Pina-D1b, and RILs with Klasic as the maternal parent and containing the *Pinb-D1b* allele (mean SKCS hardness values of 72.1 and 71.9, respectively). The final sub-population of RILs was significantly the softest and had ID377s as the maternal parent and possessed the *Pinb-D1b* allele (mean SKCS hardness value of 66.3). Both hard alleles, when present in a Klasic maternal background were harder than their counterparts in a ID377s background, indicating that the maternal background has a consistent effect on kernel texture independent of puroindoline allele. The effect of the maternal parent was not considered by either Giroux et al. (2000) or Martin et al. (2001).

Discussion

From this analysis and others (Campbell et al., 1999, 2001; Bettge & Morris, 2000; Giroux et al., 2000; Perretant et al., 2000; Martin et al., 2001), the presence of additional traits beyond the puroindoline genes that affect endosperm texture has been confirmed, specifically an effect associated with the maternal parent. Campbell et al. (1999, 2001) identified a number of regions on chromosomes other than 5DS with quantitative trait loci (QTL) having a significant effect upon endosperm texture. Perretant et al. (2000) also

identified QTLs associated with kernel texture in non-5DS regions. All these traits thus far have been encoded for by the nuclear genome of wheat. An additional source of variation specifically to kernel texture could be the cytoplasmic genomes of the mitochondria and chloroplast/amyloplast, which are inherited maternally (Kück et al., 1993). Barlow et al. (1973) observed the presence of membrane remnants around starch granules in the endosperm of wheat, and hypothesized that these remnants may represent the original amyloplast membrane of the developing starch granule. Associated with the amyloplast membrane are water-soluble pentosans, which have been shown to be associated with variation in kernel texture (Bettge & Morris, 2000). Of the two parental cultivars examined in this research, Klasic contains significantly higher ($P \leq 0.05$) concentrations of water-soluble pentosans (water-soluble pentosans per gram of whole ground wheat) than ID377s, 28.3 mg g^{-1} and 24.4 mg g^{-1}, respectively (data not shown). Anecdotal evidence suggests that variation in the maternally-inherited cytoplasmic genomes may have an effect on kernel texture. Maternally-inherited variation in grain characteristics have been observed. Millet et al. (1992) identified variation in grain weight and grain protein percentage which was inherited maternally. The maternally-inherited amyloplast is the primary candidate for the source of the maternally-inherited variation observed in this research.

To date, recombination between *Pina* and *Pinb* has only been reported in a larger population of *T. monococcum* L. segregants (1 in 395 lines) (Tranquilli et al., 1999). In hexaploid wheat, no recombination between the puroindoline genes has been observed, but populations have been smaller ($n = 83$ and $n = 139$; Giroux & Morris, 1997; and Martin et al., 2001; respectively). On the assumption that linkage is involved, the maximum genetic distance between puroindoline a and puroindoline b in the population used in this research was estimated at 1.6 centimorgans (cM) at a 95% confidence level (Table A.14A, Steel et al., 1997). In *T. monococcum*, the distance was 0.14 cM. The physical distance between puroindoline a and puroindoline b in *T. monococcum* is approximately 32 kb (Chantret et al., 2004) and approximately 30 kb in *Ae. tauschii* (Turnbull et al., 2003). Due to the colinearity of the Triticeae genomes, a similar physical distance could be assumed to occur between the two puroindoline genes in hexaploid wheat, although the genetic distances suggest otherwise. However, as it appears that wheat genes are not distributed linearly on the chromosomes, but are clustered into gene-rich regions which may be recombination hot spots (Gill et al., 1996), disparity between the physical and genetic maps can be expected. In this regard, the puroindoline genes behave like the *Glu-D1* high molecular weight (HMW) glutenin subunits (i.e. 5 + 10 and 2 + 12) in that they regularly segregate together due to their very tight linkage.

The frequency distribution of puroindoline alleles was distorted among RILs derived from ID377s as the maternal parent but not among those with Klasic as the maternal parent. The puroindoline allele frequency data contained in Giroux & Morris (1997) were analyzed and found to deviate significantly at $P = 0.05$. The allele frequency among those chromosome 5D recombinant substitution lines was 52 *Pina-D1a*/*Pinb-D1a* (soft) and 31 *Pina-D1a*/*Pinb-D1b* (hard). However, this population was a soft/hard cross. A more appropriate comparison would be the distribution of alleles in the hard/hard cross 'Butte 86'/'ND2603' (*Pina-D1b*/*Pinb-D1a* and *Pina-D1a*/*Pinb-D1b*, respectively) RIL population developed by R. Frohberg at North Dakota State University and used by Martin et al. (2001). In that population, the frequency of *Pina-D1b* versus *Pinb-D1b* alleles deviated significantly ($P = 0.01$), but in this case the *Pinb-D1b* allele was more frequent. Based on these results and the cultivar survey conducted by Morris et al. (2001), a conclusion could be drawn that there is no strong selection or other bias for either of these puroindoline alleles.

The differences in kernel texture between these two hard puroindoline alleles are much less compared to the differences between the soft, wild type allele and either of these two hard alleles. A conclusion can be drawn that the glycine to serine perturbation of the primary structure of puroindoline b and the absence of puroindoline a have dramatic (albeit unequal) effects on kernel texture. The evolutionary requirement for the puroindoline genes in *T. aestivum* is unknown. However, the absence of the puroindolines in durum wheat (*T. turgidum* ssp. *durum* [Desf.] Husn.) indicates that wheat is able to survive without them.

The present study is the first to demonstrate the importance of the maternal parent in wheat grain hardness. Further, it corroborates an earlier study that identified the 'a-null' *Pina-D1b* allele as being harder than the Gly-to-Ser 46 *Pinb-D1b* allele. The puroindoline hardness alleles are immediately available to wheat breeders for making minor modifications to grain texture within the traditional hard wheat class. Other factors, including maternal effects, will require additional research before they can be routinely utilized.

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